

## Wnt/Frizzled Signaling in the Vasculature: New Angiogenic Factors in Sight

Wnt growth factors function via Frizzled receptors to affect cellular proliferation, differentiation, apoptosis, and migration. Wnt/Frizzled signaling is now linked to human hereditary disorders with retinal vascular defects, implicating Wnts as angiogenic factors. Here, we discuss Wnts and a novel Frizzled ligand, Norrin, in physiological and pathological angiogenesis.

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Wnts are secreted, cysteine-rich glycoproteins that bind and activate Frizzled receptors, a family of seven transmembrane domain proteins. Current knowledge of Wnt signaling has been garnered from a variety of organisms, including mouse, fly, zebrafish, *Xenopus laevis*, and using mammalian cultured cells (73). Wnts govern cell proliferation, survival, differentiation, polarization, and migration by modulating both cellular and transcriptional events (49). Based on studies in mice, Wnts function during mammalian development, but Wnt signaling is also commonly altered in human cancers. The signaling cascades defined for Wnt/Frizzleds are uniquely distinct from other receptor-mediated pathways, but their diversity of cellular outputs still presents a challenge to our understanding of how Wnts work. Cellular activities governed by Wnts are also those that are critical for vascular development and angiogenesis, the process by which new blood vessels sprout from preexisting ones. Thus one might expect that Wnts would be implicated as angiogenic factors. The focus of this review is on recent studies exploring the hypothesis that Wnts are angiogenic factors and on the discovery of human retinal vascular disorders associated with Frizzleds. We will briefly overview the mechanisms of intracellular Wnt signaling, evaluate genetic and biochemical evidence that Wnts act in vascular development, and describe a novel Frizzled ligand, Norrin. These studies place Wnt/Frizzled signaling central to the development of retinal vasculature. The evidence that Wnt/Frizzled represents a novel angiogenic signaling pathway is now in sight.

### Wnts and Frizzleds: Canonical and Noncanonical Wnt Signaling Pathways

Wnts signal through Frizzleds and the transduction of the signal has been separated into a “canonical” pathway and several “noncanonical” pathways (FIGURE 1) (reviewed in Refs. 49, 54, 73). Canonical Wnt signaling involves stabilization of cytosolic  $\beta$ -catenin, turning it into a nuclear transcriptional regulator. Noncanonical signaling represents several signaling cascades activated by Wnt/Frizzleds that do not work via  $\beta$ -catenin. Both canonical and noncanonical signaling work through Frizzled activation, which involves recruit-

ment of the cytosolic Dishevelled proteins to the intracellular domain of Frizzled. After this event, the canonical and noncanonical pathways diverge in their mechanisms of action. Frizzleds contain seven transmembrane domains, an extracellular cysteine-rich domain (CRD) necessary for binding to Wnts, and an intracellular domain containing a conserved motif for Dishevelled binding (69). The low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6) function as Frizzled co-receptors in the canonical pathway, forming a ternary signaling complex on binding of Wnt to Frizzled (64).

During the resting state of canonical Wnt signaling, several cytosolic proteins form a large complex to reduce the amount of cytosolic  $\beta$ -catenin via proteolysis. This complex includes the tumor suppressor adenomatous polyposis coli (APC), which mediates the targeting of  $\beta$ -catenin to the ubiquitin-mediated proteolysis pathway. Also participating in this complex is Axin, a scaffold that brings the enzyme glycogen synthase kinase 3 and cytosolic  $\beta$ -catenin in proximity leading to phosphorylation of  $\beta$ -catenin. Activation of the canonical pathway by a Wnt results in inhibition of this turnover complex leading to increased cytosolic  $\beta$ -catenin, which then transits to the nucleus. In the nucleus,  $\beta$ -catenin associates with one of a family of Tcf/Lef transcription factors (4). Tcf/ $\beta$ -catenin complexes directly regulate the expression of numerous known target genes, many of which, such as cyclinD1 (65) and c-myc (24), are implicated in cellular proliferation. We will refer to this pathway as Wnt/ $\beta$ -catenin signaling.

Noncanonical Wnt signaling can mediate proliferation (76) and cellular movements during gastrulation in vertebrates (35) and regulate planar cell polarity (PCP) in both vertebrates (21) and invertebrates (47). Intracellular transmission of noncanonical Wnt signaling utilizes Dishevelled (2); however, there are several distinct branches of noncanonical signaling. The Wnt/PCP branch of noncanonical signaling is known to act through Rho-GTPases and JNK to regulate orientation of cellular structures (47). The Wnt/ $\text{Ca}^{2+}$  pathway utilizes G proteins or intracellular calcium influx with activation of calcium-sensitive kinases PKC and  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase II to effect Wnt-mediated signals (36). Research is still ongoing as to the function of the distinct noncanonical signaling

branches and their roles in regulating different biological phenomena. The two forms of intracellular Wnt signaling, canonical and noncanonical, conduct signaling cross talk. For instance, noncanonical Wnt signaling can antagonize Wnt/ $\beta$ -catenin signaling, a phenomenon that may occur through several mechanisms (29, 68, 70).

### Wnt Signaling Pathways and Vascular Biology

Vascular development depends on Wnt signaling, as evidenced by genetic analysis of embryos with mutations in Wnt or Frizzled genes.

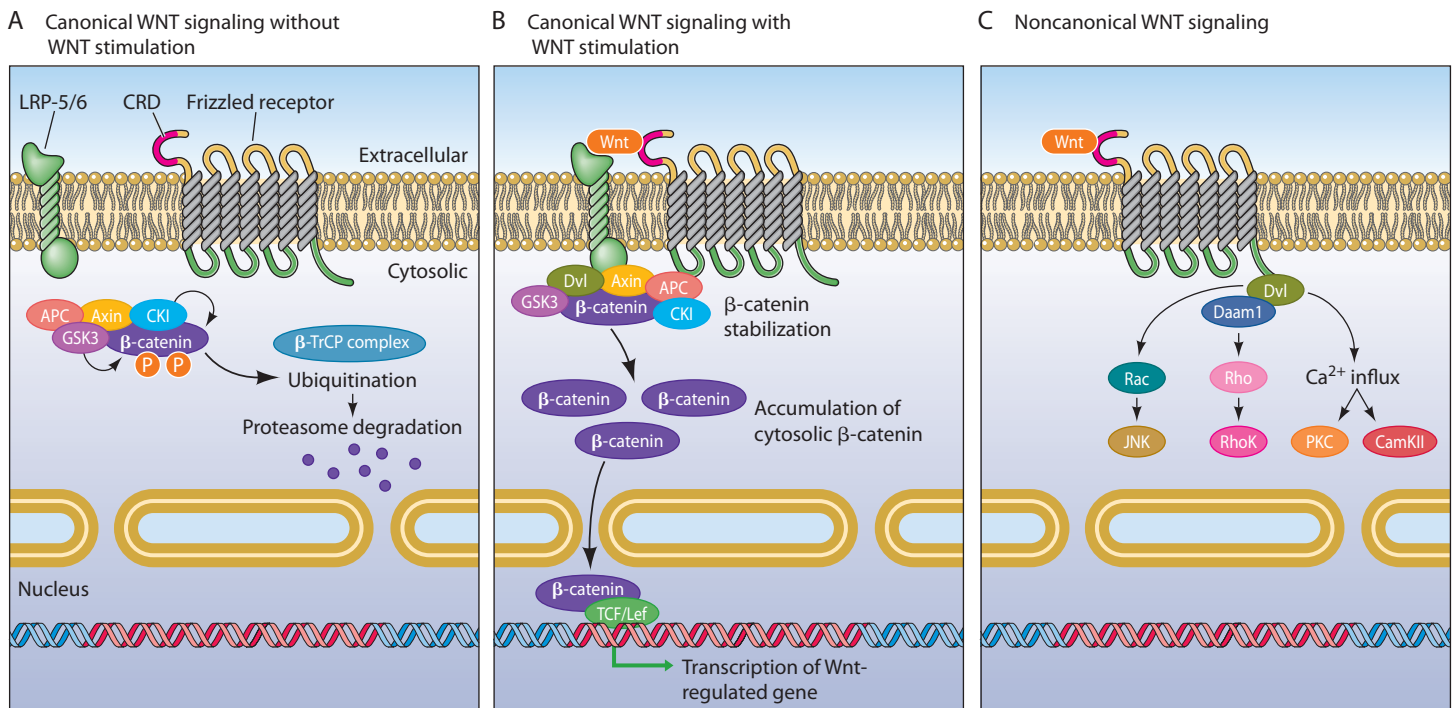
Wnt-2-deficient mouse embryos fail to establish a proper fetal capillary network in the placenta (48). Consistent with this finding is the observation that Wnt-2 is expressed in fetal vessels of the placenta (48). Thus Wnt-2, which can activate Wnt/ $\beta$ -catenin signaling (60), is implicated in the consolidation of proper placental vascularization.

Vascular formation in the mammalian gonad occurs in a sex-specific manner, during which endothelial cells migrate from the mesonephros into the gonad to form a coelomic blood vessel. Analysis of Wnt-4 knockout mice showed that Wnt-4 represses mesonephric endothelial migration in the XX gonad, preventing the

formation of male-specific coelomic blood vessels (30).

The Wnt-7b gene functions in lung vascular development, as Wnt-7b embryos bearing a gene replacement of the first exon of the Wnt-7b gene with the *lacZ* gene (Wnt-7b<sup>LacZ-/-</sup>) display severe defects in the smooth muscle component of the major pulmonary vessels (62). Mutant embryos at E18.5 had enlarged, branched vessels surrounded by extensive hemorrhage in the lungs, whereas increased cell death in vascular smooth muscle cells was detected in mutant neonates. Thus loss of Wnt-7b function results in loss of vascular smooth muscle integrity leading to pulmonary hemorrhage. Wnt-7b signals through Frizzled-1 and Frizzled-10, in cooperation with LRP5, to activate Wnt/ $\beta$ -catenin signaling (72). However, a different phenotype is associated with a Wnt-7b knockout mouse that targets the third exon. This Wnt-7b deficiency caused impaired chorion-allantois fusion during placental development but no evident vascular defects (53). Wnt-7b has also been shown to be required for the initiation of apoptosis and regression of transient ocular hyaloid vessels in mice (39).

The Frizzled-5 gene is expressed in the yolk sac, eye, and lung bud in 9.5-day-old embryos, and loss of Frizzled-5 gene function leads to death around *day 11* of embryonic development due to severe defects in yolk



**FIGURE 1. Wnt signaling transduction pathway**

A: in the canonical Wnt pathway or Wnt/ $\beta$ -catenin pathway, when signaling is at the basal state (top), cytosolic  $\beta$ -catenin is phosphorylated by GSK3 and CKI in a multiprotein complex containing the scaffold protein Axin and APC. Phosphorylated  $\beta$ -catenin is then ubiquitinated by  $\beta$ -TrCP and degraded in proteasomes. In presence of a Wnt that activates Wnt/ $\beta$ -catenin signaling (bottom), Dishevelled (Dvl) blocks the  $\beta$ -catenin degradation complex, allowing its cytosolic accumulation. Stabilized  $\beta$ -catenin then translocates into the nucleus where it associates with TCF/Lef transcription factors, leading to regulation of numerous target genes. B: in the noncanonical Wnt pathway, Dvl is linked through Daam1 to allow activation of small GTPase Rho and Rho-kinase (RhoK) and can activate JNK via Rac. In *Drosophila*, this pathway can direct cytoskeletal organization and coordinated polarization of cells in an epithelial sheet. Wnts that function in noncanonical signaling can also induce intracellular calcium flux and the activation of calcium-sensitive enzymes such as PKC and CamKII. As several other players participate in these cascades, simplified versions are schematized.

sac angiogenesis (28). Lack of Frizzled-5 reduces endothelial cell proliferation in yolk sac vasculature and, later in development, disrupts placental vasculogenesis.

Which cell types of the vasculature participate in Wnt signaling? Wnts are paracrine factors and may be produced by endothelial, mural, or epithelial cells acting on Frizzleds found on endothelial cells. Thus Wnts could conceivably be expressed from a variety of cellular sources and affect endothelial cells. Wnts and Frizzleds are endogenously expressed in both endothelial and vascular smooth muscle cells. One study reported that Wnt-5a and Frizzled-3 are expressed in human umbilical vein endothelial cells (HUVEC) and smooth muscle cells from human pulmonary artery, whereas mouse brain microvascular cells showed expression of Wnt-7a and Wnt-10b and Frizzled-1 (74). The Wnt-7a, Wnt-10b, and Wnt-13 genes and Frizzled-4, Frizzled-5, and Frizzled-6 genes are expressed in HUVEC and human dermal microvascular cells (HMVEC) (14, 44). Human umbilical vein and microvascular endothelial cells also express  $\beta$ -catenin-associated transcription factors Tcf-1, Tcf-3, Tcf-4, and Lef-1 (44). Of the Frizzleds expressed in cultured endothelial cells, Frizzled-4 and Frizzled-5 are linked to vascular development by genetic analysis in either mouse (Frizzled-5) or humans (Frizzled-4).  $\beta$ -Catenin signaling occurs in endothelial cells *in vivo*, as defined by following T-cell factor (TCF) transcriptional activity in whole animals. A  $\beta$ -catenin-activated transgenic (BAT) mouse driving expression of nuclear  $\beta$ -galactosidase reporter (BAT-gal) has been made that expresses lacZ under the control of  $\beta$ -catenin/TCF responsive elements. BAT-gal expression identifies a variety of sites of Wnt signaling, like notochord and brain, but also identifies endothelial cells as a site of  $\beta$ -catenin/TCF signaling (43).

Ectopic expression of Wnts in cultured endothelial cells can elicit biological responses, allowing a better understanding of the precise role Wnts play in the steps of angiogenesis. Pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), promote endothelial survival, proliferation, migration, and morphogenesis, and regulate the association of endothelial cells with the extracellular matrix and mural cells (20). These distinct cellular steps in angiogenesis have been evaluated in cultured endothelial cells undergoing Wnt signal activation, typically via ectopic expression of a Wnt. Mouse brain microvascular endothelial cells showed increased proliferation when Wnt-1, a canonical Wnt, was ectopically overexpressed (74). This observation was later extended to overexpressed mutant forms of  $\beta$ -catenin in a human microvascular endothelial cell line (71). Thus, as in other cell types, Wnt/ $\beta$ -catenin signaling in endothelial cells promotes proliferation. Angiogenic stimulation includes survival signals for endothelial cells, and Wnt-1/ $\beta$ -catenin signaling promotes the survival of human primary endothelial cells when cultured in low

serum (44). Activation of Wnt/ $\beta$ -catenin signaling can also promote formation of capillary-like networks developed by primary endothelial cells *in vitro*, an activity that may reflect Wnt as either a morphogenic or survival factor (44).

The action of Wnts that signal via  $\beta$ -catenin as angiogenic factors may occur through indirect means. Seven  $\beta$ -catenin/TCF binding sites occur in the gene promoter for VEGF-A (18). VEGF is upregulated as a result of mutational activation of Wnt/ $\beta$ -catenin signaling in colon cancer cells (18, 77) and in human endothelial cells (63). Gene expression changes regulated by Wnt signaling in human endothelial cells led to the identification of the interleukin-8 gene as a target of Wnt/ $\beta$ -catenin signaling in endothelial cells (44), as in other cell types (37). Interleukin-8 can induce endothelial cell proliferation, survival, and *in vitro* expression of MMP-2 and MMP-9, two matrix metalloproteinases that function in angiogenesis (38). Thus Wnt/ $\beta$ -catenin may influence endothelial cells directly or indirectly via the induction of known angiogenic factors such as VEGF and IL-8, particularly during tumor angiogenesis. A variety of other angiogenic regulators have previously been reported as Wnt target genes (Table 1), including Eph/Eprhins (3), FGF18 (61), FGF20 (11), endothelin-1 (33), Cx43 (1), uPAR (42), MMP7 (9, 15), and MMP3 (55). Thus Wnts may regulate angiogenesis through induction of multiple angiogenic genes.

Stimulation of endothelial proliferation by  $\beta$ -catenin may also be mediated by platelet endothelial cell adhesion molecule (PECAM) signaling (6). Wnt/ $\beta$ -catenin activity is implicated downstream of several distinct signaling pathways that function in endothelial cells (22, 26). Changes in localization of  $\beta$ -catenin from the membrane to the cytosol, an indicator of Wnt/ $\beta$ -catenin signaling, is found in endothelial cells during neovascularization after experimental-induced myocardial infarction (7). However, conditional inactivation of the  $\beta$ -catenin gene in endothelial cells caused increased vascular fragility during embryogenesis, likely due to an alteration of catenin/cadherin function (10).

Secreted Frizzled-related proteins (sFRPs) are a group of secreted proteins with structural homology to the extracellular CRD of Frizzled receptors. FrzA or sFRP-1, originally isolated from bovine aortic endothelium but also expressed in many other tissues, can reduce the proliferation of endothelial cells *in vitro* (17). Recombinant, purified FrzA protein can temporarily arrest growth of endothelial and smooth muscle cells *in vitro*, increasing cytosolic levels of phosphorylated  $\beta$ -catenin and decreasing levels of cyclin E and cdk2 kinase while negatively controlling angiogenesis in an ischemic muscle *in vivo* model (19). However, in chick chorioallantoic membrane and in grafted mesenchymal and glioma cells, FrzA was able to induce formation of vessels while inducing migration and tube formation of endothelial cells *in vitro* (16). This apparent contradiction highlights the

fact that the role of FrzA in vascular biology is not well understood.

### Wnt Signaling Comes into Play in Human Vascular Diseases

Impaired activity of Wnt signaling components have previously been directly linked to several human disorders, including colon and other cancers, fibrosis, tooth agenesis, and a rare disease characterized by complete absence of all four limbs called tetra-amelia (50). A direct link between Wnt signaling and a vascular phenotype in a human disorder was first reported as a connection between familial exudative vitreoretinopathy (FEVR) and mutations in the human FRIZZLED-4 gene (59). FEVR is a hereditary ocular disorder that develops with characteristic defective retinal vascularization combined with retinal detachments and leaky vasculature that bleeds and exudes. FEVR patients show a range of severity of these symptoms, with the most severe leading to blindness (41). FEVR is a genetically heterogeneous disorder, and so far four different FEVR loci have been mapped on several chromosomes, including 11q, 11p, and Xp (67). Mutations on the X chromosome linked to FEVR mapped to a novel gene referred to as the Norrie gene, sometimes referred to as the Norrie disease product (NDP) gene (12). The Norrie gene encodes for a protein product usually called Norrin and sometimes referred to as NDP, we will refer to the protein as Norrin. FRIZZLED-4 gene mutations (FIGURE 2) suggest that Wnt signaling is aberrant in FEVR. Frizzled-4 has been demonstrated to function in noncanonical Wnt signaling response in *X.*

*laevis* embryos, since Frizzled-4 can mediate PKC translocation to the plasma membrane and induction of Ca<sup>2+</sup>-calmodulin-dependent protein kinase II (59). Experimental evidence demonstrates that Norrin can bind Frizzled-4 and trigger Wnt/β-catenin signaling (75). Thus Frizzled-4 is thought to function in both Wnt/Ca<sup>2+</sup> and Wnt/β-catenin signaling. As support for Wnt signaling being important to FEVR, several FRIZZLED-4 mutant genes found in FEVR patients encode proteins that have reduced capacity to participate in noncanonical signaling (59). Several Frizzled-4 mutants were able to oligomerize with wild-type Frizzled-4, and this complex of mutant and wild-type Frizzled-4 was retained in the endoplasmic reticulum. Thus the genetic dominance of mutant Frizzled-4 in FEVR may reflect its ability to associate with wild-type Frizzled-4, blocking the capacity of endogenous Frizzled-4 to activate Wnt/β-catenin signaling (32).

The FRIZZLED-4 and the NDP genes lie at two of the four clearly identified FEVR loci. Thus other genes are also implicated in this disease. Mutations in the LRP5 gene (FIGURE 2), a known Wnt coreceptor, were described in some FEVR patients in a locus closely linked to the FRIZZLED-4 gene. Thus some mutations in the 11p region implicated in FEVR are found in the FRIZZLED-4 coding sequence and others are found in the LRP5 coding sequence. This implies that defective signaling can occur via production of mutant Frizzled-4 or LRP5 proteins (66). The identity of the genes mutated in two other FEVR loci are not known, but one may speculate that they encode players, novel or known, in Wnt signaling. It is currently estimated that mutations in one of five distinct genes can lead to

**Table 1. Target genes induced by Wnt/β-catenin signaling**

Target Gene	Refs.	Description	Function
EphB2/EphB3/Ephrin-B1	3	Receptors for ephrins and ephrin ligand	Vascular remodeling: cell-cell communication
VEGF	74	Vascular endothelial growth factor	Endothelial cell permeability, network formation: vascular permeability
FGF	11, 59	Fibroblast growth factor	Regulator of angiogenesis
EDN-1	32	Endothelin-1	Vascular cell maintenance
IL-8	36, 42	Interleukin-8	Endothelial cell proliferation and survival, network formation, regulation of MMPs
Cx43	1	Connexin43	Gap-junction communication: vasculogenesis and vascular remodeling
uPAR	40	Urokinase-type plasminogen activator receptor	Regulation of endothelial cell migration
MMP7	9, 15	Matrix metalloproteinase-7	Degradation of extracellular matrix: cell migration
MMP3	53	Matrix metalloproteinase-3	Degradation of extracellular matrix: cell migration

Target genes induced by Wnt/β-catenin signaling that encode known angiogenic regulators. The target genes were drawn from a comprehensive list provided by the Wnt homepage at <http://www.stanford.edu/~rnusse/wntwindow.html>.

manifestation of the FEVR phenotype in humans (67).

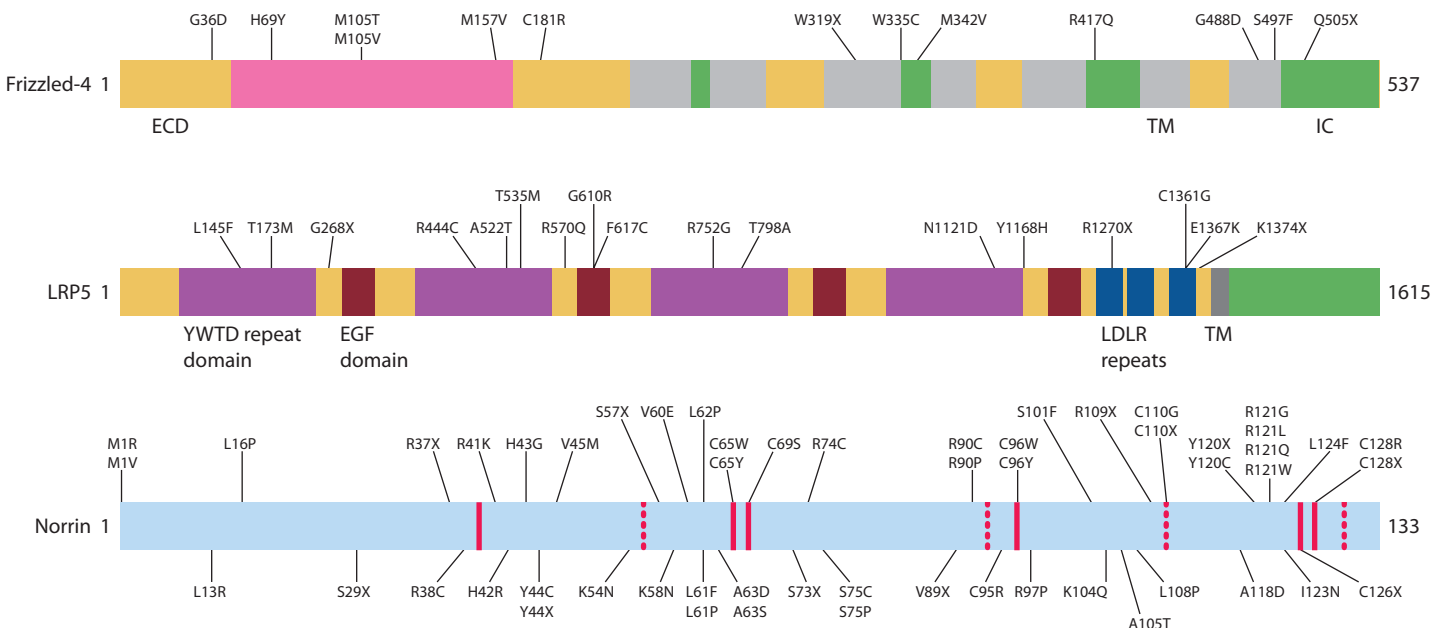
In agreement with the defects observed in FEVR, retinas from neonatal *Frizzled-4*<sup>-/-</sup> mutant mice were found to be completely devoid of the two intraretinal capillary beds (75). The *Frizzled-4* mutant phenotype includes enlarged and tortuous major arteries and veins of the retina, and arteriolar arborization is diminished. Additionally, the nerve fiber layer shows a higher number of small fenestrated vessels at the vitreal face of the retina, which extend perpendicularly toward the inner retina. In the normal mouse, the hyaloid vasculature represents a set of vessels existing after birth in the vitreous body that completely regress by P17. The hyaloid vasculature is still present at the same stage in *Frizzled-4*<sup>-/-</sup> mouse, and retinal hemorrhages are common in these mice. Our studies demonstrate that *Frizzled-4* is expressed in adult murine retinal vasculature (FIGURE 3).

### Norrin, a New Non-Wnt Ligand of Frizzled

As its name suggests, the NDP gene has more commonly been associated with a human disorder termed Norrie disease (ND). Genetic linkage studies localized the NDP gene to the short arm of the X chromosome (8), and this led to the cloning and characterization of the NDP gene (5, 13). ND is an X-linked congenital retinal dysplasia involving blindness at birth and, sometimes, deafness and mental retardation.

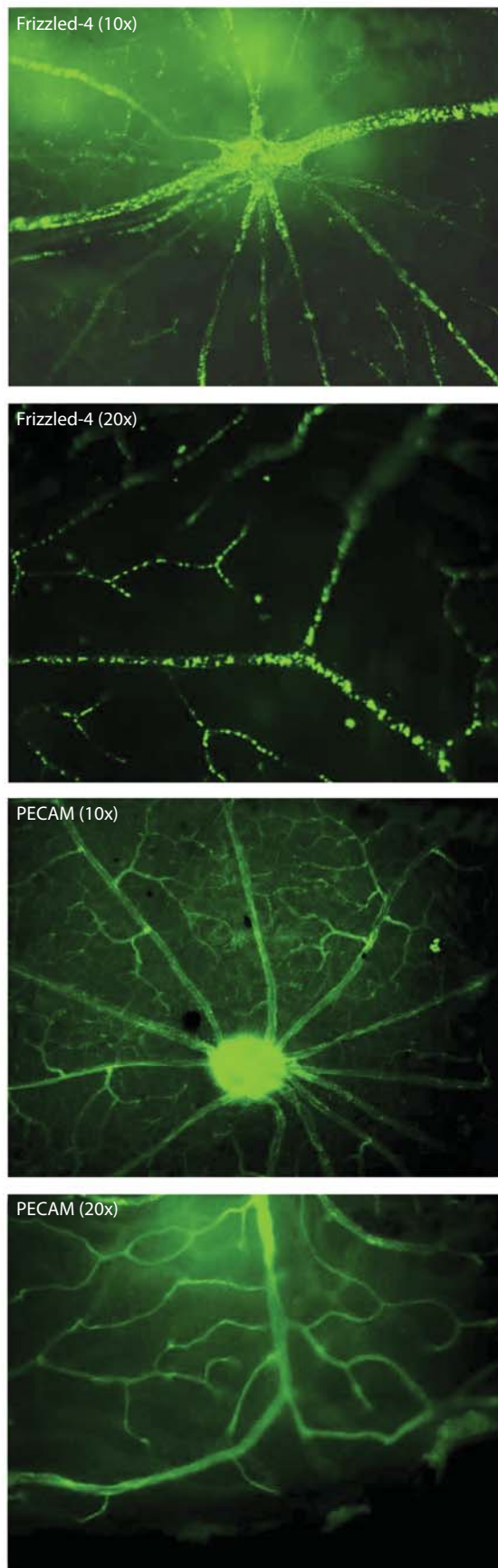
Originally described by Heine and Norrie in the 1930s (50a) and intensively studied by Warburg in the 1960s (72a, 72b), ND involves retinal detachment and retinal folds, vitreous opacity, and other symptoms that make it clinically similar to FEVR, except that ND can be associated with mental retardation and deafness.

Norrin, the protein encoded by the NDP gene, is a cysteine-rich secreted product that belongs to the superfamily of growth factors containing a cysteine knot motif (45). Other members of the cysteine knot superfamily are transforming growth factor-β (TGF-β) and nerve growth factor (NGF). Genetic analysis in ND patients has revealed a wide variety of missense, nonsense, deletion, and splice-site mutations in the NDP gene. The reported mutations in ND patients number in excess of 100, and different mutations in the Norrin gene have been reported in patients with FEVR, retinopathy of prematurity (ROP), and Coats' disease. A schematic diagram of most characterized point mutations in Norrin are shown in FIGURE 2. Most of the mutations in patients either affect a normal cysteine residue of Norrin or amino acids in the vicinity of cysteine residues. These cysteines are well preserved in the superfamily of proteins to which Norrin belongs and may be crucial in protein tertiary structure. Although Norrin has no sequence homology with Wnts, the high degree of similarity in vascular phenotypes between the *Frizzled-4*<sup>-/-</sup> mutant mouse and the defects reported for the *Ndph*<sup>-/-</sup> mutant mice (57, 58) led investigators to further analyze the hypothesis that



**FIGURE 2. Schematic diagram of Frizzled-4, LRP5, and Norrin proteins showing mutations within protein domains reported in ND, FEVR, ROP, or Coats' disease cases**

Only known nucleotide missense/nonsense substitutions were included. Transmembrane domains are depicted in gray, intracellular domains are in light green, and cysteine residues are represented by red lines. Extracellular domains (ECD) are represented in yellow. Frizzled-4: cysteine-rich domain (CRD) domain is depicted as a pink color box, part of the full ECD. Transmembrane domains are represented as described previously (34). LRP5: the four portions of the ECD, including the five YWTD repeats (YWTD domain), are in purple, EGF domains are in dark brown, and LDLR repeats are in blue, with numbering as described in Ref. 25. Norrin: red dotted lines indicate position of cysteine residues involved in disulphide bridges. Other conserved cysteine residues in the cysteine knot domain are depicted in full red lines.



**FIGURE 3.** Whole-mount immunofluorescence staining of adult mouse retinas for Frizzled-4 or PECAM, a known marker for endothelium. Venal and arterial vasculature can be clearly seen ( $\times 10$ ), as well as microcapillaries ( $\times 20$ ), showing the presence of Frizzled-4 in vasculature of mouse retinas.

Norrin might be a Frizzled-4 ligand (75). Norrin showed high specificity of binding and affinity for Frizzled-4 *in vitro*. In addition, Norrin coexpression with Frizzled-4 led to activation of Wnt/ $\beta$ -catenin signaling in 293 cells, and several mutants of Norrin were unable to function in this assay. Thus, despite the lack of homology to Wnts, Norrin functions like a Wnt, binding the CRD domain of Frizzled-4 with nanomolar affinity and acting through a Frizzled and LRP co-receptor (73). Recently, it has been shown that Norrin is necessary for the proper regression of hyaloid vessels in mice after birth (52) as regression is delayed in mice expressing a truncated form of Norrin (58), as well as in *Lrp5<sup>-/-</sup>* mice (31) and *Frizzled-4<sup>-/-</sup>* mutant mice (75). Regression of hyaloid vasculature is partially mediated by macrophages, and a delay of hyaloid regression can affect subsequent retinal angiogenesis. The effects on retinal vasculature in FEVR or ND might thus be a direct consequence of impaired macrophage or hyaloid vascular cell function or secondary to hypoxia that develops. The defective retinal angiogenesis may also arise from a lack of normal functional Norrin in the outer retina alone or increased oxygen levels in the vitreous due to the extended presence of hyaloid vessels. These are important issues to resolve before proceeding with potential therapeutic intervention of the finely balanced development of hyaloid and retinal vessels.

Norrin has been initially described as a potential angiogenic factor for retinal vessels. Based on several observations, Norrin's function might not be strictly limited to the eye vasculature. Expression analysis showed significant levels of Norrin transcripts, not only in specific layers of the retina of human, mouse, and rabbit but also in cerebellum, hippocampus, olfactory bulb, and cortex of rabbit brain (23). An intriguing observation is the peripheral venous insufficiency reported in association with ND in a large Costa Rican family and in an isolated case in Great Britain (46, 56). That is, some Norrin mutations may lead to vascular defects in the venous system beyond the eye. Finally, homozygous mutation of the murine Norrin gene (*Ndph*) presents many features present in human ND. Loss of murine Norrin also leads to incomplete decidualization during pregnancy and defective vascular development of the decidua, leading to infertility (40). Thus there is a potential role for Norrin in female reproductive angiogenesis. Of note, a recently reported *Frizzled-4<sup>-/-</sup>* mutant mouse is also infertile, with non-functional corpora lutea probably due to impaired angiogenesis (27). The fact that, as opposed to *Frizzled-4<sup>-/-</sup>* mice, the *Ndph<sup>-/-</sup>* mice have functional corpora lutea indicates that Norrin, although expressed in the ovarian tissue, may not be the only mediator of Frizzled-4 signaling in the mouse ovaries. This opens the possibility of other angiogenic Frizzled-4 ligands yet to be determined. An important area for future study is to establish how widespread Frizzled-4 function as an angiogenic receptor beyond the retina.

Although therapeutic intervention in ND or FEVR is far from a reality, a light of hope is apparent based on Norrin. As proof of principle, a transgenic mouse model that ectopically expresses Norrin in *Ndph*<sup>-/-</sup> mutant mouse background partially restores the formation of normal retinal vascular network and retinal neuronal function. Norrin protein could similarly be applied or expressed in mutant eyes, setting the stage for evaluation of its therapeutic potential (51).

### Concluding Remarks

The concept that Wnts and Norrin are a new class of angiogenic factor is gaining support. Clearly, Wnt/Frizzled signaling is critical for embryonic vascular development, and Norrin/Frizzled signaling is critical for angiogenesis in the developing eye. Whether angiogenic Wnt activities are mediated by Wnt/ $\beta$ -catenin or noncanonical Wnt signaling, or both, is still unresolved. Evidence exists for the involvement of both classes of Wnt signaling in angiogenesis. The first human developmental disorder linked to Wnt/Frizzled signaling was in fact FEVR, which was linked to Frizzled-4. The discovery of new FEVR loci may further implicate the Wnt pathway in retinal angiogenesis, and mouse modeling will likely be used to explore how prevalent Wnt/Frizzled signaling is in developmental and physiological angiogenesis. Because of these exciting discoveries, the field of Wnt/Norrin/Frizzled signaling in angiogenesis is now clearly visible. ■

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